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TECHNICAL NOTE

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The Differential Elution of Drugs from XAD-2 Resin

In a previously published XAD-2 resin method for the extraction of drugs from biofluids and tissue [*1*] the elution of the drug(s) from the resin was accomplished with one or more organic solvents. Drugs exhibiting acid, basic, and neutral characteristics were eluted from the column in the same fraction. Preliminary drug screening was usually carried out by thin-layer chromatography (TLC), employing a spray sequence which can achieve detection of almost all the commonly encountered drugs. This procedure of drug detection and identification requires that the analyst be especially competent in utilizing a spray sequence effectively. A preliminary study was conducted to determine whether the drugs eluted differentially from XAD-2 resin. Herein is presented a technique in which drugs are separated physically into acid or neutral, and basic drug fractions.

Materials and Methods

Columns and Samples

Columns were prepared from glass and filled to a height of 15 cm with XAD-2 resin (about 20 g). Samples of biofluids and tissue were prepared prior to extraction with XAD-2 resin as previously described [*1*].

Extraction Procedure

Biological fluids and tissue samples were passed through the XAD-2 resin bed for removal of drugs. The differential elution of the adsorbed drugs from the resin was performed by sequential elution of the drugs in four consecutive steps. A volume of 0.05*M* sodium acetate buffer, pH 4.55, was first run through the resin bed and discarded. Drugs exhibiting acidic or neutral characteristics were then eluted from the column with 100 ml of chloroform in 20-ml aliquots. The column was then washed with 30 ml of 0.01*M* potassium carbonate, pH 11.6, which was discarded. The drugs exhibiting basic characteristics were removed from the resin bed by washing with 100 ml chloroform:isopropanol (3:1) in 20-ml aliquots. Two or three drops of 0.1% HCl in methanol were added to each of the organic solvent fractions and the solvents evaporated under an air stream.

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Thin-Layer Chromatography

The residue was dissolved in two drops of 95% ethanol and spotted on Silica Gel G plates (coated to a thickness of 0.25 mm). Acid and neutral drugs were spotted and sprayed separately from basic drugs. The plates were developed 10 cm in either of two solvent systems, the composition of these systems being ethyl acetate:methanol:ammonium hydroxide (85:10:5) or chloroform:methanol:ammonium hydroxide (90:10:1).

Spray Reagents

Plates spotted with the acid and neutral drug fraction were sprayed mainly with the following reagents.

Mercurous Nitrate—One gram of mercurous nitrate was dissolved in 49 ml of water and 1 ml of concentrated nitric acid. The acid solution was then diluted to 100 ml with water. Barbiturates, glutethimide, and diphenylhydantoin were detected with this spray.

Dragendorff's Reagent—If drugs such as diazepam or oxazepam are suspected, the plate is sprayed with Dragendorff's reagent (see basic drug reagents) for localization of these compounds at this point.

Trinder's Reagent—Four grams of mercurous chloride were dissolved in 85 ml of water. To this solution were added 12 ml of 1*N* hydrochloric acid and 4 g of ferric nitrate. The solution was then diluted to 100 ml with water. Salicylamide was detected with this reagent.

Caffeine Spray Reagent—Caffeine was located by spraying the plate first with a mixture of 1% iodine plus 1% potassium iodine in 95% ethanol, then with 25% HCl in water:95% ethanol (50:50).

Phenacetin Spray Reagent—Phenacetin was detected by spraying with a solution of 5% potassium ferricyanide:10% ferric chloride:distilled water (1:2:8).

Plates spotted with the basic drug fraction were sprayed mainly with the following reagents.

Ninhydrin—Ninhydrin (0.4 g) was dissolved in 100 ml of acetone. After spraying, the plates were placed under ultraviolet (UV) light for 5 min for localization of amphetamine.

Iodoplatinate Reagent—Potassium iodide (4.5 g) was dissolved in 45 ml of water and then mixed with 5 ml of 5% platinic chloride. The mixture was then diluted to 150 ml with water. This spray was used to detect basic drugs and narcotics.

Dragendorff's Reagent—A stock solution was prepared by dissolving 20 g of potassium iodide in 50 ml of water. To the solution were added 12.5 g of bismuth subnitrate, 25 ml of glacial acetic acid, and 100 ml of water. The spray reagent was prepared from 1 part stock solution, 2 parts glacial acetic acid, and 3 parts water. This spray was utilized mainly for the localization of methaqualone.

Recovery Data

Percent recoveries were done by UV spectrophotometry. Blood samples were spiked with the smallest amount of drug necessary to give an optical density value ranging from 0.50 to 0.75.

Results and Discussion

After blood and urine samples were spiked with various acid, basic, and neutral drugs, they were passed through the XAD-2 resin. A preliminary attempt was made to elute these drugs differentially from the resin. The two solvent fractions were analyzed by TLC to determine which of the drugs, if any, were in fact eluted and in which fraction they would appear. Table 1 shows the elution characteristics of the various acid, neutral,

TABLE 1—*Elution characteristics of acid, neutral, and basic drugs from XAD-2 resin (0.5 μg/ml).*

Acid Fraction		Basic Fraction	
Amobarbital	Carisoprodal	Cocaine	Chlordiazepoxide
Butabarbital	Diazepam	Codeine	Chlorpromazine
Mephobarbital	Oxazepam	Meperidine	Meprobamate
Methohexital		Methadone	Thioridazine
	Glutethimide		
Pentobarbital		Morphine	
	Methaqualone		Ephedrine
Secobarbital			
	Methyprylon	Amphetamine	
			Methapyrilene
Caffeine		Methamphetamine	
	Diphenylhydantoin		
Theophylline			Propoxyphene
		Quinine	
	Salicylamide		
Warfarin			

and basic drugs investigated. It is apparent that nearly all the characteristically acid and neutral drugs were eluted in the acid-chloroform fraction and the basic drugs in the basic-chloroform:isopropanol fraction. It is interesting to note that meprobamate and carisoprodol (which is an isopropyl derivative of meprobamate) are both extractable from acid and alkaline solutions; however, on resin extraction the meprobamate appears in the basic fraction and the carisoprodol in the acid fraction. Another fact worthy of comment is that diazepam and oxazepam are eluted in the acid fraction. Although these drugs are usually classed as basic drugs they do, in fact, exhibit neutral drug behavior during pH-dependent solvent extractions; that is, they are readily extracted from both acid and alkaline aqueous solutions. The differential elution technique has a distinct advantage in that each drug appears in one solvent fraction and does not distribute itself through both the acid and alkaline fractions.

In Table 2 the percent recoveries of several drugs were determined from the differential elution of the XAD-2 resin. Acid, neutral, and basic drugs which are commonly encountered in biological materials were chosen. Large amounts of some drugs were used since quantitation was done by UV spectrophotometry, where low extinction coefficients do not permit useful optical density values when a small amount of drug is analyzed. Under these conditions the recoveries seem adequate, except for methaqualone and morphine.

TABLE 2—*Percent recovery of drugs added to blood.*

Drug	Recovery, %	Amount Added,[a] μg/ml
Amobarbital	84.2	29.9
Phenobarbital	89.5	59.1
Secobarbital	87.4	30.9
Caffeine	85.7	12.9
Methapyrilene	95.3	12.1
Codeine	88.8	120.2
Morphine	59.8	144.7
Methaqualone	55.9	4.6

[a] Quantitation by UV spectrophotometry.

Efforts to improve the recovery of these drugs are presently being continued. Eluting solvents other than chloroform and chloroform:isopropanol (3:1) may be more fruitful, for example, Bastos et al [2] elute acid drugs with isopropyl ether.

Finally, in order to show that a reasonably small quantity of drug could be detected in this manner, a number of blood samples were spiked with various drugs, differentially eluted from the XAD-2 resin, and analyzed by TLC. Sensitivity of detection was limited to 0.5 μg/ml for all drugs tested, as shown in Table 3.

Previously, XAD-2 resin was used in this laboratory to extract drugs from biological materials by concentrating acid, basic, and neutral drugs in one fraction. This occasionally posed a problem in drug identification by TLC. Either a complicated sequence of spray reagents had to be used to detect the presence of these drugs or two separate plates had to be spotted, one being sprayed only for acid and neutral drugs and the other for basic drugs. Manipulation of a complicated spray sequence requires an especially competent and experienced analyst. The differential elution of the XAD-2 resin eliminates some of the difficulties, and drugs can be detected and identified more readily by TLC without maximum expertise in TLC technique.

TABLE 3—*Drugs detected by TLC in concentration of 0.5 μg/ml after differential elution.*

Amobarbital	Codeine
Butabarbital	Meperidine
Butalbital	Methadone
Mephobarbital	Morphine
Pentobarbital	
	Glutethimide
Phenobarbital	
	Methaqualone
Secobarbital	
	Diphenylhydantoin
Chlordiazepoxide	
Chlorpromazine	Quinine
Diazepam	Salicylamide
Oxazepam	
Thioridazine	Warfarin

Summary

Biological fluids and tissue extracts prepared according to a previously published method were passed through a column of Amberlite® XAD-2 resin for removal of drugs. The differential elution of the adsorbed drugs from the resin was performed by sequential elution of the drugs in four steps. The column was first washed with 30 ml of 0.05*M* sodium acetate buffer, pH 4.55. Drugs exhibiting acidic or neutral characteristics were then eluted from the column with 100 ml of chloroform in 20-ml aliquots. The column was then washed with 30 ml of 0.1*M* potassium carbonate, which was discarded. Drugs exhibiting basic characteristics were then eluted from the column with 100 ml of chloroform:isopropanol (3:1) in 20-ml aliquots.

Sensitivity of drug detection with this method by thin-layer chromatography was 0.5 μg/ml in a 10-ml sample for nearly all drugs tested.

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